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=> fil hcaplu
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FILE COVERS 1907 - 23 Jul 2003  VOL 139 ISS 4
FILE LAST UPDATED: 22 Jul 2003  (20030722/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.


=> d stat que
L1          5 SEA FILE=REGISTRY ABB=ON  HEPATITIS A?/CN
L2        127 SEA FILE=REGISTRY ABB=ON  DNASE/BI
L3          2 SEA FILE=REGISTRY ABB=ON  PRONASE/BI
L4      16957 SEA FILE=REGISTRY ABB=ON  PROTEINASE/BI
L5       2448 SEA FILE=HCAPLUS ABB=ON  L1 OR HEPATITIS(W)A OR HAV
L6      20551 SEA FILE=HCAPLUS ABB=ON  L2 OR DNASE
L7       7914 SEA FILE=HCAPLUS ABB=ON  L3 OR PRONASE
L8     145351 SEA FILE=HCAPLUS ABB=ON  L4 OR PROTEINASE
L11       295 SEA FILE=HCAPLUS ABB=ON  L5 (L) (L6 OR L7 OR L8 OR GRISEUS(2W)T
                RYPSIN)
L12       190 SEA FILE=HCAPLUS ABB=ON  L11 AND (PRODUCT? OR PURIF? OR
                ISOLAT? OR MANUF? OR PREP? OR PREPN OR CHARACTER?)
L13         1 SEA FILE=HCAPLUS ABB=ON  L12 AND VERO


=> d ibib abs hitrn l13

L13  ANSWER 1 OF 1  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:        2003:472410  HCAPLUS
DOCUMENT NUMBER:         139:51598
TITLE:                   Method of production of purified
                         hepatitis A virus particles, and their use in vaccine
                         preparation
INVENTOR(S):             Tauer, Christa; Meyer, Heidi; Mitterer, Artur;
                         Barrett, Noel
PATENT ASSIGNEE(S):      Baxter Healthcare S.A., Switz.
SOURCE:                  PCT Int. Appl., 40 pp.
                         CODEN: PIXXD2
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  1
PATENT INFORMATION:

     PATENT NO.       KIND  DATE          APPLICATION NO.  DATE
```

WO 2003049766	A2	20030619	WO 2002-EP14008	20021210	
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003124511	A1	20030703	US 2001-6205	20011210	

PRIORITY APPLN. INFO.: US 2001-6205 A 20011210

AB The present invention provides methods of **purifn.** of hepatitis A virus (HAV) from the supernatant of an infected cell culture by filtering and virus inactivation treatment and prodn. of a **prepn.** of **purified** HAV antigen under serum-free conditions. Contaminating impurities which might derive from the cells or the cell culture medium are efficiently removed by the method of invention. The invention is also directed to an HAV vaccine compn. comprising a **prepn.** consisting of **purified** mature HAV particles in an amt. sufficient to induce a protective immune response. The vaccine of present invention was compared in regards to its immunogenicity with 2 com. vaccines (VAQTA 50U and HAVRIX 1440). The antibody titers of the pooled sera of mice given the undiluted vaccine of invention at 15-20 IU/mL were 3541 mIU/mL compared to 2541 mIU/mL and 691 mIU/mL when given undiluted VAQTA and HAVRIX, resp.

IT **9001-92-7**, Protease
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(microbial; **prepn.** of **purified hepatitis A** virus particles and their use in vaccine **prepn.**)

IT **9003-98-9, DNase 9036-06-0, Pronase**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**prepn.** of **purified hepatitis A** virus particles and their use in vaccine **prepn.**)

```
=> d stat que
L1            5 SEA FILE=REGISTRY ABB=ON  HEPATITIS A?/CN
L2          127 SEA FILE=REGISTRY ABB=ON  DNASE/BI
L3            2 SEA FILE=REGISTRY ABB=ON  PRONASE/BI
L4        16957 SEA FILE=REGISTRY ABB=ON  PROTEINASE/BI
L5         2448 SEA FILE=HCAPLUS ABB=ON  L1 OR HEPATITIS(W)A OR HAV
L6        20551 SEA FILE=HCAPLUS ABB=ON  L2 OR DNASE
L7         7914 SEA FILE=HCAPLUS ABB=ON  L3 OR PRONASE
L8       145351 SEA FILE=HCAPLUS ABB=ON  L4 OR PROTEINASE
L11         295 SEA FILE=HCAPLUS ABB=ON  L5 (L) (L6 OR L7 OR L8 OR GRISEUS(2W)T
                 RYPSIN)
L12         190 SEA FILE=HCAPLUS ABB=ON  L11 AND (PRODUCT? OR PURIF? OR
                 ISOLAT? OR MANUF? OR PREP? OR PREPN OR CHARACTER?)
L13           1 SEA FILE=HCAPLUS ABB=ON  L12 AND VERO
L15          42 SEA FILE=HCAPLUS ABB=ON  L11 AND (VACCIN? OR PARTICLE? OR
                 MATURE(W)VIRUS)
L16          41 SEA FILE=HCAPLUS ABB=ON  L15 NOT L13
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Searched by M. Smith

=> d ibib abs hitrn l16 1-41

L16 ANSWER 1 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:173635 HCAPLUS
DOCUMENT NUMBER: 138:216487
TITLE: A method of sequestering a protein in a complex to simplify purification by manufacture as a fusion protein with polymerizing protein
INVENTOR(S): Tillett, Daniel; Thomas, Torsten
PATENT ASSIGNEE(S): Protigene Pty. Ltd., Australia
SOURCE: PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003018616	A1	20030306	WO 2002-AU1159	20020827

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: AU 2001-7298 A 20010827
AB A method of manufg. a protein in an expression host that simplified purifn. without the need for extensive chromatog. or affinity chromatog. purifn. is described. The method involves manufg. the protein as a fusion protein with a carrier that forms homopolymers. The protein can be purified by capture with the unmodified form of the homopolymer-forming protein. The fusion protein can be hydroyzed with a proteinase specific for a linker peptide connecting the two moieties. Methods of using the FtsZ protein of Escherichia coli as the carrier moiety are demonstrated.
IT **97162-88-4, Proteinase** 3C
RL: CAT (Catalyst use); USES (Uses)
(fusion protein contg. cleavage site for; method of sequestering protein in complex to simplify purifn. by manuf. as fusion protein with polymg. protein)
IT **97162-88-4DP, Proteinase** 3C, fusion products with FtsZ protein
RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(prepn. and purifn. of; method of sequestering protein in complex to simplify purifn. by manuf. as fusion protein with polymg. protein)
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 2 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:937303 HCAPLUS
DOCUMENT NUMBER: 138:20443
TITLE: Endocrine disruptor screening using DNA chips of

endocrine disruptor-responsive genes
INVENTOR(S): Kondo, Akihiro; Takeda, Takeshi; Mizutani, Shigetoshi; Tsujimoto, Yoshimasa; Takashima, Ryokichi; Enoki, Yuki; Kato, Ikunoshin
PATENT ASSIGNEE(S): Takara Bio Inc., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 386 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002355079	A2	20021210	JP 2002-69354	20020313

PRIORITY APPLN. INFO.: JP 2001-73183 A 20010314
JP 2001-74993 A 20010315
JP 2001-102519 A 20010330

AB A method and kit for detecting endocrine-disrupting chems. using DNA microarrays are claimed. The method comprises prepg. a nucleic acid sample contg. mRNAs or cDNAs originating in cells, tissues, or organisms which have been brought into contact with a sample contg. the endocrine disruptor. The nucleic acid sample is hybridized with DNA microarrays having genes affected by the endocrine disruptor or DNA fragments originating in these genes have been fixed. The results obtained are then compared with the results obtained with the control sample to select the gene affected by the endocrine disruptor. Genes whose expression is altered by tri-Bu tin, 4-octaphenol, 4-nonylphenol, di-N-Bu phthalate, dichlorohexyl phthalate, octachlorostyrene, benzophenone, diethylhexyl phthalate, diethylstilbestrol (DES), and 17-.beta. estradiol (E2), were found in mice by DNA chip anal.

IT **97162-88-4, Proteinase** 3C
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(semaphorin 3C, sema domain, Ig domain, short basic domain secreted; endocrine disruptor screening using DNA chips of endocrine disruptor-responsive genes)

L16 ANSWER 3 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:785977 HCAPLUS
TITLE: Quality of therapeutic plasma-requirements for marketing authorization
AUTHOR(S): Heiden, Margarethe; Seitz, Rainer
CORPORATE SOURCE: Paul-Ehrlich-Institut, Langen, D-63225, Germany
SOURCE: Thrombosis Research (2002), 107(Suppl. 1), S47-S51
CODEN: THBRAA; ISSN: 0049-3848
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Fresh frozen plasma (FFP) contains higher levels of intact coagulation factors and coagulation and fibrinolysis inhibitors than solvent/detergent-treated plasma (SD plasma), and also greater residual cell contamination. SD plasma is a **particle**-free plasma of uniform quality. SD treatment, however, has the specific result of reducing the activities of some inhibitors. Both plasma types carry a minimal residual risk of transmitting human immunodeficiency virus (HIV)-1/2, hepatitis virus B (HBV), and hepatitis virus C (HCV), but SDP is, in addn., also safe with respect to other lipid-enveloped viruses and perhaps with respect to hepatitis virus A (**HAV**), also due to its antibody (Ab) content.Future revisions of therapeutic plasma safety and

quality stds. should consider the following points:For FFP:reduce residual cell count in all FFP units to values below 5.times.106 leukocytes/l;screen donors for Parvovirus B19 genome and antibodies in order to establish a sufficiently large collection of genome-neg. and antibody-pos. donors whose FFP can be used for selected patients;For SDP:introduce pool testing for Parvovirus B19 genome; fix an upper limit for genome and a lower limit for antibody content;in addn. to the std. quality control methods for therapeutic plasma, focus on assays to test for functionally intact **proteinase** inhibitors such as .alpha.2antiplasmin (.alpha.2AP) and .alpha.1proteinase inhibitor (.alpha.1PI) that are important for plasma indications. Com. available kits may not be sufficient to show changes in inhibition kinetics.For both types:introduce an activation marker such as thrombin-antithrombin complex (TAT) as a random test to monitor activation processes during withdrawal, sepn., manufg., and storage;abolish inappropriate parameters like Antithrombin III (AT III) and coagulation factor XI that are not relevant for changes in plasma quality;finally, support every effort towards establishing an efficient documentation and reporting system on efficacy and side effects of plasma transfusions. Effective reporting alone might help to reveal deficiencies of specific plasma quality and to overcome them through modifications to manufg. processes and testing, or by defining its indications more precisely.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:536899 HCAPLUS
DOCUMENT NUMBER: 137:229123
TITLE: Analysis of deletion mutants indicates that the 2A polypeptide of hepatitis A virus participates in virion morphogenesis
AUTHOR(S): Cohen, Lisette; Benichou, Daniele; Martin, Annette
CORPORATE SOURCE: Unite de Genetique Moleculaire des Virus Respiratoires, URA CNRS 1966, Institut Pasteur, Paris, 75724, Fr.
SOURCE: Journal of Virology (2002), 76(15), 7495-7505
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Unlike all other picornaviruses, the primary cleavage of the **hepatitis A** virus (**HAV**) polyprotein occurs at the 2A/2B junction and is carried out by the only **proteinase** encoded by the virus, 3Cpro. The resulting P1-2A capsid protein precursor is subsequently cleaved by 3Cpro to generate VP0, VP3, and VP1-2A, which assoc. as pentamers. An unidentified cellular **proteinase** acting at the VP1/2A junction releases the mature capsid protein VP1 from VP1-2A later in the morphogenesis process. Although these aspects of polyprotein processing are well characterized, the function of 2A is unknown. To study its role in the viral life cycle, we assessed the infectivity of synthetic, genome-length RNAs contg. 11 different in-frame deletions in the 2A region. Deletions in the N-terminal 40% of 2A abolished infectivity, whereas deletions in the C-terminal 60% resulted in viruses with a small-focus replication phenotype. C-terminal deletions in 2A had no effect on RNA replication kinetics under 1-step growth conditions, nor did they have an effect on capsid protein synthesis and 3Cpro-mediated processing. However, C-terminal deletions in 2A altered the VP1/2A cleavage, resulting in accumulation of uncleaved VP1-2A precursor in virions and possibly accounting for a delay in the appearance of

infectious **particles** with these mutants, as well as a 4-fold decrease in specific infectivity of the virus **particles**. When the capsid proteins were expressed from recombinant **vaccinia** viruses, the N-terminal part of 2A was required for efficient cleavage of the P1-2A precursor by 3Cpro and assembly of structural precursors into pentamers. These data indicate that the N-terminal domain of 2A must be present as a C-terminal extension of P1 for folding of the capsid protein precursor to allow efficient 3Cpro-mediated cleavages and to promote pentamer assembly, after which cleavage at the VP1/2A junction releases the mature VP1 protein, a process that appears to be necessary to produce highly infectious **particles**.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:475422 HCAPLUS
DOCUMENT NUMBER: 137:333663
TITLE: Pretreatment to avoid positive RT-PCR results with inactivated viruses
AUTHOR(S): Nuanualsuwan, Suphachai; Cliver, Dean O.
CORPORATE SOURCE: Department of Population Health and Reproduction, University of California, School of Veterinary Medicine, Davis, CA, 95616-8743, USA
SOURCE: Journal of Virological Methods (2002), 104(2), 217-225
CODEN: JVMEDH; ISSN: 0166-0934
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteric viruses that are important causes of human disease must often be detected by reverse transcription-polymerase chain reaction (RT-PCR), a method that commonly yields pos. results with samples that contain only inactivated virus. This study was intended to develop a pretreatment for samples, so that inactivated viruses would not be detected by the RT-PCR procedure. Model viruses were human **hepatitis A** virus, **vaccine** poliovirus 1 and feline calicivirus as a surrogate for the Norwalk-like viruses. Each virus was inactivated (from an initial titer of .apprxeq.103 PFU/mL) by UV light, hypochlorite or heating at 72.degree.. Inactivated viruses, that were treated with **proteinase** K and RNase for 30 min at 37.degree. before RT-PCR, gave a neg. result, which is to say that no amplicon was detected after the reaction was completed. This antecedent to the RT-PCR method may be applicable to other types of viruses, to viruses inactivated in other ways and to other mol. methods of virus detection.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:115241 HCAPLUS
DOCUMENT NUMBER: 136:291491
TITLE: Hepatitis A virus polyprotein processing by Escherichia coli proteases
AUTHOR(S): Pinto, Rosa M.; Guix, Susana; Gonzalez-Dankaart, Juan F.; Caballero, Santiago; Sanchez, Gloria; Guo, Ke-Jian; Ribes, Enric; Bosch, Albert
CORPORATE SOURCE: Department of Microbiology, University of Barcelona, Barcelona, 08028, Spain
SOURCE: Journal of General Virology (2002), 83(2), 359-368
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hepatitis A virus (HAV) encodes a single polyprotein, which is post-translationally processed. This processing represents an essential step in capsid formation. The virus possesses only one protease, 3C, responsible for all cleavages, except for that at the VP1/2A junction region, which is processed by cellular proteases. In this study, data demonstrates that HAV polyprotein processing by Escherichia coli protease(s) leads to the formation of particulate structures. P3 polyprotein processing in E. coli is not dependent on an active 3C protease: the same processing pattern is obsd. with wild-type 3C or with several 3C mutants. However, this processing pattern is temp.-dependant, since it differs at 37 or 42.degree.C. The bacterial protease(s) cleave scissile bonds other than those of HAV; this contributes to the low efficiency of **particle** formation.
IT **9001-92-7**, Protease
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**hepatitis A** virus polyprotein processing by Escherichia coli proteases)
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:22878 HCAPLUS
DOCUMENT NUMBER: 136:195858
TITLE: Poliovirus 3C Protease-Mediated Degradation of Transcriptional Activator p53 Requires a Cellular Activity
AUTHOR(S): Weidman, Mary K.; Yalamanchili, Padmaja; Ng, Bryant; Tsai, Weimin; Dasgupta, Asim
CORPORATE SOURCE: Department of Microbiology, Immunology, and Molecular Genetics, UCLA School of Medicine, University of California, Los Angeles, Los Angeles, CA, 90095-1747, USA
SOURCE: Virology (2001), 291(2), 260-271
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Infection of HeLa cells with poliovirus leads to rapid shut-off of host cell transcription by RNA polymerase II. Previous results have suggested that both the basal transcription factor TBP (TATA-binding protein) and transcription activator proteins such as CREB (cAMP-responsive element-binding protein) and Oct-1 (the octamer-binding factor) are cleaved by the viral-encoded protease, 3CPro. Here we demonstrate that the transcriptional activator (and tumor suppressor) p53 is degraded by the viral protease 3C both in vivo and in vitro. Unlike other transcription factors that are directly cleaved by 3Cpro, degrdn. of p53 requires a HeLa cell activity in addn. to 3CPro. The degrdn. of p53 by 3CPro does not appear to involve the ubiquitin pathway of protein degrdn. **Vaccinia** virus infection of HeLa cells leads to inactivation of the cellular activity required for 3CPro-mediated degrdn. of p53. The **vaccinia**-encoded protein (CrmA) is known to inhibit caspase I (ICE protease) that converts inactive IL-1.beta. to an active secreted form. Incubation of HeLa cells with caspase I inhibitor Z-VAD-fmk does not interfere with 3CPro-mediated degrdn. of p53. The cellular activity present in exts. of HeLa cells can be fractionated through phosphocellulose. A partially purified fraction that elutes at 0.6 M KCl from phosphocellulose contains the activity that degrades p53 in a

3CPro-dependent manner. These results suggest that both poliovirus-encoded protease 3CPro and a cellular activity are required for the degrdn. of p53 obsd. in cells infected with poliovirus. (c) 2001 Academic Press.

IT **97162-88-4**, 3C Protease
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(poliovirus 3C protease-mediated degrdn. of transcriptional activator p53 requires a cellular activity)

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:10302 HCAPLUS
DOCUMENT NUMBER: 136:74555
TITLE: **Vaccine** against foot-and-mouth disease
INVENTOR(S): King, Andrew; Burman, Alison; Audonnet, Jean-Christophe; Lombard, Michel
PATENT ASSIGNEE(S): Merial, Fr.
SOURCE: PCT Int. Appl., 79 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000251	A1	20020103	WO 2001-FR2042	20010627
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
FR 2810888	A1	20020104	FR 2000-8437	20000629
EP 1294400	A1	20030326	EP 2001-949547	20010627
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
BR 2001012071	A	20030520	BR 2001-12071	20010627

PRIORITY APPLN. INFO.: FR 2000-8437 A 20000629
WO 2001-FR2042 W 20010627

OTHER SOURCE(S): MARPAT 136:74555

AB The invention concerns a **vaccine** against foot-and-mouth disease, using as antigen an efficient amt. of empty capsids of the foot-and-mouth virus, said empty capsids being obtained by expressing, in eukaryotic cells, cDNA of the P1 region of the foot-and-mouth virus genome coding for the capsid and cDNA of the region of the foot-and-mouth virus genome coding for protease 3C, the **vaccine** further comprising a carrier or excipient pharmaceutically acceptable in veterinary medicine. The invention also concerns the insertion of a mutation in the sequence VP2 (introducing a cysteine), thereby stabilizing the empty capsids and the resulting viruses.

IT **97162-88-4**, **Proteinase** 3C
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(cDNA encoding; **vaccine** against foot-and-mouth disease)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 9 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:221363 HCAPLUS
DOCUMENT NUMBER: 135:370309
TITLE: Detection of antibodies to **HAV** 3C **proteinase** in experimentally infected chimpanzees and in naturally infected children
AUTHOR(S): Kabrane-Lazizi, Y.; Emerson, S. U.; Herzog, C.; Purcell, R. H.
CORPORATE SOURCE: Laboratory of Infectious Diseases, Hepatitis Viruses and Molecular Hepatitis Sections, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Vaccine (2001), 19(20-22), 2878-2883
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Com. assays for the diagnosis of **hepatitis A** detect antibody to **hepatitis A** virus (anti-**HAV**), but they cannot discriminate between antibody resulting from infection and antibody induced by inactivated **vaccine**. With the licensing and increasing use of inactivated **hepatitis A** **vaccines**, there is a need for a test to distinguish between infection and **vaccination**. Since antibodies to viral non-structural proteins are elicited by infection but not by **vaccination** with inactivated **vaccine**, the authors developed and evaluated a test for such antibodies. The antibody response to the non-structural 3C **proteinase** (anti-3C) of virus **HAV** was studied by ELISA in chimpanzees exptl. infected with virulent (wild type) or with attenuated **HAV** strains and in children who received inactivated **HAV vaccine** or placebo during a **vaccination** trial in Nicaragua. Anti-3C was detected in 89% of 18 chimpanzees infected with wild-type **HAV** strains and 27% of 26 chimpanzees infected with attenuated **HAV** strains. There was a direct correlation between severity of hepatitis and magnitude of the anti-3C response. In the **vaccine** trial, anti-3C was detected only in children who were infected with **HAV** during the study; IgG anti-3C persisted for at least 15 mo after infection in one child. **Vaccinated** and uninfected children remained neg. for anti-3C. The anti-3C response can be regarded as an indicator of viral replication. Its detection should be useful for distinguishing between antibody acquired in response to **HAV** infection and antibody induced by immunization with inactivated **vaccine**.
IT **97162-88-4**, 3C **Proteinase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ELISA of antibodies to **hepatitis A** virus 3C **proteinase** in exptl. infected chimpanzees and in naturally infected children)
REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 10 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:146666 HCAPLUS
DOCUMENT NUMBER: 136:18946
TITLE: Immune responses and protection against foot-and-mouth

disease virus (FMDV) challenge in swine **vaccinated** with adenovirus-FMDV constructs
AUTHOR(S): Mayr, G. A.; O'Donnell, V.; Chinsangaram, J.; Mason, P. W.; Grubman, M. J.
CORPORATE SOURCE: North Atlantic Area, Agriculture Research Service, Department of Agriculture, Plum Island Animal Disease Center, Greenport, NY, 11944-0848, USA
SOURCE: Vaccine (2001), 19(15-16), 2152-2162
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A replication-defective adenovirus 5 encoding foot-and-mouth disease virus (FMDV) capsid and 3C proteinase coding regions (Ad5-FMDV3CWT) was used to **vaccinate** swine. A single inoculation utilizing 1.times.108 plaque forming units (pfu) or an inoculation of 1.times.108 followed by a boost of 5.times.108 pfu Ad5-FMDV3CWT were tested, along with an inoculation and boost using an adenovirus encoding the FMDV capsid coding region and an inactive form of the 3C proteinase (Ad5-FMDV3CMUT). Sera collected from these animals were examd. for the presence of FMDV-specific antibodies using immunopptn., neutralization, and ELISA assays specific for IgM, IgG1 and IgG2. Efficacy studies were performed by placing the **vaccinated** swine in contact with an FMDV-infected swine and monitoring for signs of disease and changes in serum antibody levels. Ad5-FMDV3CMUT, which is unable to produce FMDV capsid structures, did not elicit FMDV-neutralizing antibodies or protect against FMD. Single inoculation with Ad5-FMDV3CWT generated FMDV-specific neutralizing antibodies, and reduced clin. signs in challenged swine, but failed to completely protect the majority of swine from FMD. Swine which received a primary **vaccination** with Ad5-FMDV3CWT followed by the boost at 4 wk generated high levels of FMDV-neutralizing antibodies resulting in complete protection of 5 of the 6 swine and limited disease in the remaining animal. Increased efficacy of the two-dose regimen was assocd. with heightened levels of FMDV-specific IgG1 and IgG2 antibodies.

IT **97162-88-4**, 3C **Proteinase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(immune response to foot-and-mouth disease virus challenge in swine **vaccinated** with adenovirus-FMDV constructs)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 11 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:278094 HCAPLUS
DOCUMENT NUMBER: 132:307247
TITLE: Hepatitis A **vaccines**
INVENTOR(S): D'Hondt, Erik
PATENT ASSIGNEE(S): Smithkline Beecham Biologicals S.A., Belg.
SOURCE: PCT Int. Appl., 26 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023574	A2	20000427	WO 1999-EP7765	19991008
WO 2000023574	A3	20000727		

W: CA, JP, US

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
EP 1121420 A2 20010808 EP 1999-952573 19991008
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
JP 2002527105 T2 20020827 JP 2000-577285 19991008
PRIORITY APPLN. INFO.: GB 1998-22714 A 19981016
WO 1999-EP7765 W 19991008

AB A process for the prodn. of inactivated Hepatitis A virus substantially free of host cell contamination is described, the process comprising: (a) culturing Hepatitis A virus and harvesting a hepatitis A prepn.; (b) treating said hepatitis A prepn. with a protease; and thereafter (c) sepg. intact virus from protease-digested material; (d) inactivating said virus. Also described are **vaccines** comprising the inactivated hepatitis A virus, preferably in combination with strong adjuvants.

IT **9001-92-7**, Protease
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(digestion; **hepatitis A vaccines** comprising host cell contamination-free/inactivated **hepatitis A** virus and immune adjuvant)

L16 ANSWER 12 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:183040 HCAPLUS
DOCUMENT NUMBER: 132:319659
TITLE: Evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion
AUTHOR(S): Martin, Javier; Dunn, Glynis; Hull, Robin; Patel, Varsha; Minor, Philip D.
CORPORATE SOURCE: Division of Virology, National Institute for Biological Standards and Control, Potters Bar, EN6 3QG, UK
SOURCE: Journal of Virology (2000), 74(7), 3001-3010
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 20-yr-old female hypogammaglobulinemic patient received monotypic Sabin 3 **vaccine** in 1962. The patient excreted type 3 poliovirus for a period of 637 days without developing any symptoms of poliomyelitis, after which excretion appeared to have ceased spontaneously. The evolution of Sabin 3 throughout the entire period of virus excretion was studied by characterization of seven sequential isolates from the patient. The isolates were analyzed in terms of their antigenic properties, virulence, sensitivity for growth at high temps., and differences in nucleotide sequence from the Sabin type 3 **vaccine**. The isolates followed a main lineage of evolution with a rate of nucleotide substitution that was very similar to that estd. for wild-type poliovirus during person-to-person transmission. There was a delay in the appearance of antigenic variants compared to sequential type 3 isolates from healthy **vaccines**, which could be one of the possible explanations for the long-term excretion of virus from the patient. The distribution of mutations in the isolates identified regions of the virus possibly involved in adaptation for growth in the human gut and virus persistence. None of the isolates showed a full reversion of the attenuated and temp.-sensitive phenotypes of Sabin 3. Information of this sort will help in the assessment of the risk of spread of virulent polioviruses from long-term excretors and in the design of therapies to stop long-term

excretion. This will make an important contribution to the decision-making process on when to stop **vaccination** once wild poliovirus has been eradicated.

IT **97162-88-4, Proteinase** 3C
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion)

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 13 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:749547 HCAPLUS
DOCUMENT NUMBER: 132:75774
TITLE: Improving proteolytic cleavage at the 3A/3B site of the hepatitis A virus polyprotein impairs processing and **particle** formation, and the impairment can be complemented in trans by 3AB and 3ABC
AUTHOR(S): Kusov, Yuri; Gauss-Muller, Verena
CORPORATE SOURCE: Institute for Medical Microbiology and Hygiene, Medical University of Lubeck, Lubeck, D-23538, Germany
SOURCE: Journal of Virology (1999), 73(12), 9867-9878
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The orchestrated liberation of viral proteins by 3Cpro-mediated proteolysis is pivotal for gene expression by picornaviruses. Proteolytic processing is regulated either by the amino acid sequence at the cleavage site of the substrate or by cofactors covalently or noncovalently linked to the viral **proteinase**. To det. the role of the amino acid sequence at cleavage sites 3A/3B and 3B/3C that are essential for the liberation of 3Cpro from its precursors and to assess the function of the stable processing intermediates 3AB and 3ABC, we studied the effect of cleavage site mutations on **hepatitis A** virus (**HAV**) polyprotein processing, **particle** formation, and replication. Using the recombinant **vaccinia** virus system, we showed that the normally retarded cleavage at the 3A/3B junction can be improved by altering the amino acid sequence at the scissile bond such that it matches the preferred **HAV** 3C cleavage sites. In contrast to the processing products of the wild-type polyprotein, 3ABC was no longer detectable in the mutant. VP0 and VP3 were generated less efficiently, implying that processing of the structural protein precursor P1-2A depends on the presence of stable 3ABC and/or 3AB. In addn., cleavage of 2BC was impaired in 3AB/3ABC-deficient mutants. Formation of **HAV particles** was not affected in mutants with blocked 3A/3B and/or 3B/3C cleavage sites. However, 3ABC-deficient mutants produced small nos. of **HAV particles**, which could be augmented by coexpressing 3AB or 3ABC. The hydrophobic domain of 3A that has been proposed to mediate membrane anchorage of the replication complex was crucial for restoration of defective **particle** formation. In vitro transcripts of the various cleavage site mutants were unable to initiate an infectious cycle, and no progeny viruses were obtained even after blind passages. Taken together, the data suggest that accumulation of uncleaved **HAV** 3AB and/or 3ABC is pivotal for both viral replication and efficient **particle** formation.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 14 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:697572 HCAPLUS
DOCUMENT NUMBER: 132:34410
TITLE: Development of replication-defective adenovirus serotype 5 containing the capsid and 3C protease coding regions of foot-and-mouth disease virus as a **vaccine** candidate
AUTHOR(S): Mayr, Gregory A.; Chinsangaram, Jarasvech; Grubman, Marvin J.
CORPORATE SOURCE: Plum Island Animal Disease Center, USDA, ARS, NAA, Greenport, NY, 11944, USA
SOURCE: Virology (1999), 263(2), 496-506
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A recombinant replication-defective human adenovirus serotype 5 vector contg. FMDV capsid, P1-2A, and viral 3C protease coding regions was constructed. Two viral clones were isolated, Ad5-P12X3CWT, contg. the wild-type (WT) 3C protease that processes capsid polyprotein precursor into mature capsid proteins, and Ad5-P12X3CMUT, contg. a point mutation in the protease coding region that inhibits processing. In 293 cells infected with either virus, synthesis of the FMDV capsid polyprotein precursor occurred, but processing of the polyprotein into structural proteins VP0, VP3, and VP1 occurred only in 3CWT virus-infected cells. Immunopptn. with monospecific and monoclonal antibodies indicates possible higher order structure formation in Ad5-P12X3CWT virus-infected cells. The viruses were used to elicit immune responses in mice inoculated i.m. Only virus contg. the 3CWT elicited a neutralizing antibody response. After boosting, this neutralizing antibody response increased. Swine inoculated i.m. with Ad5-P12X3CWT virus developed a neutralizing antibody response and were either completely or partially protected from contact challenge with an animal directly inoculated with virulent FMDV. This adenovirus vector may be an efficient system for the delivery of FMDV cDNA into animals, leading to a high level of neutralizing antibody prodn. and protection from FMDV challenge. (c) 1999 Academic Press.
IT **97162-88-4**, 3C Protease
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(protective immune response induced by replication-defective adenovirus expressing capsid and 3C protease genes of foot-and-mouth disease virus)
REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 15 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:520139 HCAPLUS
DOCUMENT NUMBER: 131:298402
TITLE: Development of DNA **vaccines** for foot-and-mouth disease, evaluation of **vaccines** encoding replicating and non-replicating nucleic acids in swine
AUTHOR(S): Beard, C.; Ward, G.; Rieder, E.; Chinsangaram, J.; Grubman, M. J.; Mason, P. W.
CORPORATE SOURCE: Agricultural Research Service, North Atlantic Area, Plum Island Animal Disease Center, United States Department of Agriculture, Greenport, NY, USA

SOURCE: Journal of Biotechnology (1999), 73(2,3), 243-249
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors have developed naked DNA **vaccine** candidates for foot-and-mouth disease (FMD), an important disease of domestic animals. The virus that causes this disease, FMDV, is a member of the picornavirus family, which includes many important human pathogens, such as poliovirus, **hepatitis A** virus, and rhinovirus. Picornaviruses are characterized by a small (7-9000 nucleotide) RNA genome that encodes capsid proteins, processing proteinases, and enzymes required for RNA replication. The authors have developed 2 different types of DNA **vaccines** for FMD. The first DNA **vaccine**, pP12X3C, encodes the viral capsid gene (P1) and the processing **proteinase** (3C). Cells transfected with this DNA produce processed viral antigen, and animals inoculated with this DNA using a gene gun produced detectable antiviral immune responses. Mouse inoculations with this plasmid, and with a deriv. contg. a mutation in the 3C **proteinase**, indicated that capsid assembly was essential for induction of neutralizing antibody responses. The second DNA **vaccine** candidate, pWRMHX, encodes the entire FMDV genome, including the RNA-dependent RNA polymerase, permitting the plasmid-encoded viral genomes to undergo amplification in susceptible cells. PWRMHX encodes a mutation at the cell binding site, preventing the replicated genomes from causing disease. Swine inoculated with this **vaccine** candidate produce viral **particles** lacking the cell binding site, and neutralizing antibodies that recognize the virus. Comparison of the immune responses elicited by pP12X3C and pWRMHX in swine indicate that the plasmid encoding the replicating genome stimulated a stronger immune response, and swine inoculated with pWRMHX by the i.m., intradermal, or gene gun routes were partially protected from a highly virulent FMD challenge.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 16 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:456423 HCAPLUS
DOCUMENT NUMBER: 131:211419
TITLE: Maturation of the **hepatitis A** virus capsid protein VP1 is not dependent on processing by the 3Cpro **proteinase**
AUTHOR(S): Martin, Annette; Benichou, Daniele; Chao, Shih-Fong; Cohen, Lisette M.; Lemon, Stanley M.
CORPORATE SOURCE: Unite de Virologie Moleculaire, URA CNRS 1966, Institut Pasteur, Paris, 75724, Fr.
SOURCE: Journal of Virology (1999), 73(8), 6220-6227
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Most details of the processing of the **hepatitis A** virus (**HAV**) polyprotein are known. Unique among members of the family Picornaviridae, the primary cleavage of the **HAV** polyprotein is mediated by 3Cpro, the only **proteinase** known to be encoded by the virus, at the 2A/2B junction. All other cleavages of the polyprotein have been considered to be due to 3Cpro, although the precise location and mechanism responsible for the VP1/2A cleavage have been controversial. Here we present data that argue strongly against the involvement of the **HAV** 3Cpro **proteinase** in the

maturation of VP1 from its VP1-2A precursor. Using a heterologous expression system based on recombinant **vaccinia** viruses directing the expression of full-length or truncated capsid protein precursors, we show that the C terminus of the mature VP1 capsid protein is located near residue 764 of the polyprotein. However, a proteolytically active **HAV** 3Cpro that was capable of directing both VP0/VP3 and VP3/VP1 cleavages in **vaccinia** virus-infected cells failed to process the VP1-2A precursor. Using site-directed mutagenesis of an infectious mol. clone of **HAV**, we modified potential VP1/2A cleavage sites that fit known 3Cpro recognition criteria and found that a substitution that ablates the presumed 3Cpro dipeptide recognition sequence at Glu764-Ser765 abolished neither infectivity nor normal VP1 maturation. Altered electrophoretic mobility of VP1 from a viable mutant virus with an Arg764 substitution indicated that this residue is present in VP1 and that the VP1/2A cleavage occurs downstream of this residue. These data indicate that maturation of the **HAV** VP1 capsid protein is not dependent on 3Cpro processing and may thus be uniquely dependent on a cellular **proteinase**.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 17 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:150567 HCAPLUS
DOCUMENT NUMBER: 130:279123
TITLE: Intrinsic signals for the assembly of hepatitis A virus **particles**. Role of structural proteins VP4 and 2A
AUTHOR(S): Probst, Christian; Jecht, Monika; Gauss-Muller, Verena
CORPORATE SOURCE: Institute of Medical Microbiology and Hygiene, Medical University of Lubeck, Lubeck, 23538, Germany
SOURCE: Journal of Biological Chemistry (1999), 274(8), 4527-4531
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Capsid assembly is the final event of virus replication, and its understanding is pivotal for the design of empty capsid-based recombinant **vaccines** and drug delivery systems. Although the capsid structure of several members of the picornavirus family has been elucidated, little is known about the structural elements governing the assembly process that is tightly assocd. with proteolytic processing of the viral polyprotein. Among the picornaviruses, hepatitis A virus (HAV) is unique in that it contains VP1-2A as a structural component and the small structural protein VP4, which argues for an assembly pathway different from that proposed for other picornaviruses. Using a recombinant system we show here that proteolytic processing of the HAV capsid proteins' precursor P1-2A is independent of the terminal domains 2A and VP4 of the substrate. However, both terminal domains play distinct roles in the assembly of viral **particles**. 2A as part of P1-2A is a primary signal for the assembly of pentameric structures which only further aggregate to empty viral capsids when VP4 is present as the N terminus of the precursor. **Particle** formation in the hepatovirus genus is thus regulated by two intrinsic signals that are distinct from those described for other picornaviruses.

IT **97162-88-4**, 3C **Proteinase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)

(role of structural proteins VP4 and 2A as intrinsic signals for the assembly of **hepatitis A** virus **particles**.)
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 18 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:127030 HCAPLUS
DOCUMENT NUMBER: 130:178352
TITLE: An expression vector derived from replication-competent Sabin type I poliovirus for use in oral mucosal **vaccines**
INVENTOR(S): Bae, Yong Soo; Jung, Hye Rhan
PATENT ASSIGNEE(S): Altwell Biotech. Inc., S. Korea
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9907859	A1	19990218	WO 1998-KR242	19980807
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9887512	A1	19990301	AU 1998-87512	19980807
EP 966538	A1	19991229	EP 1998-938993	19980807
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000503551	T2	20000328	JP 1999-511995	19980807
BR 9806084	A	20010918	BR 1998-6084	19980807

PRIORITY APPLN. INFO.: KR 1997-37812 A 19970807
WO 1998-KR242 W 19980807

AB A replication-competent recombinant Sabin type 1 poliovirus vector contg. a sequence coding for multiple cloning site and 3C-protease cleavage site is provided. This vector makes it easy to introduce various **vaccine** genes from infectious viruses to the Sabin 1 poliovirus, and facilitates to produce chimeric Sabin 1 polioviruses that are expected to be powerful oral mucosal **vaccines** against several infectious viral diseases. The antigen is cleaved from the viral polyprotein during normal maturation, liberating it into the cytoplasm and allowing the virus to assemble and to propagate. Construction of a virus cDNA with a 3C protease cleavage site between amino acids 1 and 2 of the polyprotein is described. Only one of the constructs (clone pTZ-PVS-3m) showed a plating efficiency comparable to the wild type virus. The gene for p24gag of HIV-1 was inserted at the multicloning site and the virus propagated from transcribed RNA. Plating efficiency was retained and the virus directed the formation of p24 detectable by radioimmunopptn. The viral RNA contg. the p24gag gene was stable over 12 passages in culture. Similar results were found with gp120env.

IT **97162-88-4**, 3C Protease
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(cleavage site for; expression vector derived from replication-competent Sabin type I poliovirus for use in oral mucosal **vaccines**)
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 19 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:810182 HCAPLUS
DOCUMENT NUMBER: 130:165308
TITLE: Membrane permeability induced by hepatitis A virus proteins 2B and 2BC and proteolytic processing of HAV 2BC
AUTHOR(S): Jecht, Monika; Probst, Christian; Gauss-Muller, Verena
CORPORATE SOURCE: Institute for Medical Microbiology and Hygiene, Medical University of Lubeck, Lubeck, 23538, Germany
SOURCE: Virology (1998), 252(1), 218-227
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The ability to rearrange membranes is a unique feature of nonstructural proteins 2B, 2C, and 2BC of some picornaviruses. To analyze in detail membrane binding of the resp. proteins of **hepatitis A** virus (**HAV**), they were transiently expressed in the **vaccinia**/T7 system, and their effect on membrane permeability was studied using .beta.-galactosidase as reporter. Although 2C had no effect, the significantly increased reporter activity obsd. in the extracellular space of 2B- and 2BC-expressing cells points to a specific effect of **HAV** proteins 2B and 2BC on membrane permeability. In biochem. fractionation studies, **HAV** 2C and 2BC showed properties of integral membrane proteins, whereas 2B was assocd. with membranes as a peripheral protein. **Proteinase** 3C-mediated cleavage of precursor 2BC in vivo was most efficient when the enzyme was coexpressed in its precursor forms P3 or 3ABC, which both include the membrane-anchoring domain 3A. The 3ABC showed the same soly. pattern as 2BC, suggesting that colocalization of 2BC and 3ABC might be required for the efficient liberation of 2B and 2C and occurs on membranes that have been proposed as the site of viral RNA replication. (c) 1998 Academic Press.
REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 20 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:620248 HCAPLUS
DOCUMENT NUMBER: 129:313293
TITLE: Processing of **proteinase** precursors and their effect on **hepatitis A** virus **particle** formation
AUTHOR(S): Probst, Christian; Jecht, Monika; Gauss-Muller, Verena
CORPORATE SOURCE: Institute for Medical Microbiology, Medical University of Lubeck, Lubeck, 23538, Germany
SOURCE: Journal of Virology (1998), 72(10), 8013-8020
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteolytic processing of the picornaviral polyprotein mediated by the differential action of virus-encoded **proteinase**(s) is pivotal to both RNA genome replication and capsid formation. Possibly to enlarge the

array of viral proteins, picornaviral polyprotein processing results in intermediate and mature products which apparently have distinct functions within the viral life cycle. For **hepatitis A** virus (**HAV**), we report here on the autoproteolysis of precursor polypeptides comprising the only viral **proteinase**, 3Cpro, and on their role in viral **particle** formation. Following transient expression of a nested set of 3Cpro-contg. proteins (P3, 3ABC, 3BCD, 3CD, 3BC, and 3C) in eukaryotic cells, the extent of processing was detd. by analyzing the cleavage products. The 3C/3D site was more efficiently cleaved than those at the 3A/3B and 3B/3C sites, leading to the accumulation of the intermediate product 3ABC. In the absence of 3A from the precursor, cleavage at the 3B/3C site was further reduced and a switch to an alternative 3C/3D site was obsd. Coexpression of various parts of P3 with the precursor of the viral structural proteins P1-2A showed that all 3C-contg. intermediates cleaved P1-2A with almost equal efficiency; however, viral **particles** carrying the neutralizing epitope form much more readily in the presence of the complete P3 domain than with parts of it. These data support the notion that efficient liberation of structural proteins from P1-2A is necessary but not sufficient for productive **HAV** capsid formation and suggest that the polypeptides flanking 3Cpro promote the assembly of viral **particles.**

IT **97162-88-4**, Protease 3Cpro
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(processing of **proteinase** precursors and their effect on hepatitis virus **particle** formation)

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 21 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:266469 HCAPLUS
DOCUMENT NUMBER: 129:26759
TITLE: Antibody response in mice inoculated with DNA expressing foot-and-mouth disease virus capsid proteins
AUTHOR(S): Chinsangaram, Jarasvech; Beard, Clayton; Mason, Peter W.; Zellner, Marla K.; Ward, Gordon; Grubman, Marvin J.
CORPORATE SOURCE: Plum Island Animal Disease Center, Agricultural Research Service, USDA, Greenport, NY, 11944, USA
SOURCE: Journal of Virology (1998), 72(5), 4454-4457
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Candidate foot-and-mouth disease (FMD) DNA **vaccines** designed to produce viral capsids lacking infectious viral nucleic acid were evaluated. Plasmid DNAs contg. a portion of the FMDV genome coding for the capsid precursor protein (P1-PA) and wild-type or mutant viral proteinase 3C (plasmids P12X3C or P12X3C-mut, resp.) were constructed. Cell-free translation reactions programmed with pP12X3C (wild-type 3C) and pP12X3C-mut produced a capsid precursor, but only the reactions programmed with the plasmid encoding the functional proteinase resulted in P1-2A processing and capsid formation. Baby hamster kidney (BHK) cells also produced viral capsid proteins when transfected with these plasmids. Plasmid P12X3C was administered to mice by i.m., intradermal, and epithelial (gene gun) inoculations. Anti-FMD virus (FMDV) antibodies were detected by radioimmunopptn. (RIP) and plaque redn. neutralization assays only in sera of mice inoculated by using a gene gun. When pP12X3C and

pP12X3C-mut were inoculated into mice by using a gene gun, both plasmids elicited an antibody response detectable by RIP but only pP12X3C elicited a neutralizing antibody response. These results suggest that capsid formation in situ is required for effective immunization. Expression and stimulation of an immune response was enhanced by addn. of an intron sequence upstream of the coding region, while addn. of the FMDV internal ribosome entry site or leader proteinase (L) coding region either had no effect or reduced the immune response.

IT **97162-88-4, Proteinase** 3C
RL: BSU (Biological study, unclassified); BIOL (Biological study) (antibody response in mice inoculated with DNA expressing foot-and-mouth disease virus capsid proteins)

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 22 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1997:625646 HCAPLUS
DOCUMENT NUMBER: 127:219540
TITLE: Methods for detecting antibodies to **HAV** 3C **proteinase**
INVENTOR(S): Stewart, Denneen; Schultheiss, Tina; Purcell, Robert H.; Emerson, Suzanne U.
PATENT ASSIGNEE(S): Government of the United States of America, Represented by the Secretary, Department of Health and Human Services, USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9734136	A2	19970918	WO 1997-US3428	19970313
WO 9734136	A3	19980205		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2247177	AA	19970918	CA 1997-2247177	19970313
AU 9723180	A1	19971001	AU 1997-23180	19970313
EP 888549	A2	19990107	EP 1997-915863	19970313
EP 888549	B1	20000503		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
AT 192579	E	20000515	AT 1997-915863	19970313
US 6156499	A	20001205	US 1998-142239	19980903

PRIORITY APPLN. INFO.: US 1996-13333P P 19960313
WO 1997-US3428 W 19970313

AB The present invention discloses methods for detecting antibodies to **HAV** 3C **proteinase**. These methods can distinguish an individual with a natural infection from one who has been **vaccinated** with an inactivated **vaccine** and are thus of utility in the diagnosis of **hepatitis A** in situations

in which **vaccination** is widespread.
IT **97162-88-4**, 3C **Proteinase**
RL: ANT (Analyte); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(methods for detecting antibodies to **HAV** 3C **proteinase**)

L16 ANSWER 23 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:613432 HCAPLUS
DOCUMENT NUMBER: 127:276797
TITLE: Detection of antibodies to the nonstructural 3C **proteinase** of **hepatitis A** virus
AUTHOR(S): Stewart, Deneen R.; Morris, Tina S.; Purcell, Robert H.; Emerson, Suzanne U.
CORPORATE SOURCE: Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892-0740, USA
SOURCE: Journal of Infectious Diseases (1997), 176(3), 593-601
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Hepatitis A** virus (**HAV**) infection can stimulate the prodn. of antibodies to structural and nonstructural proteins of the virus. However, **vaccination** with an inactivated **vaccine** produces antibodies exclusively to the structural proteins. Current diagnostic assays, such as the Abbott HAVAB test used to det. exposure to **HAV**, detect antibodies only to the structural proteins and as a result are not able to distinguish between a natural infection and **vaccination** with an inactivated virus. Therefore, an ELISA was developed that is specific for antibodies to the nonstructural protein 3C of **HAV** and thus serves to document the occurrence of viral replication. Antibodies to the **proteinase** were not detected by this assay in serum from HAVAB-seropos. primates that were immunized with inactivated **HAV**. However, antibodies to the **proteinase** were detected in the serum of all primates exptl. infected with virulent **HAV** and in the serum of naturally infected humans.
IT **97162-88-4**, 3C **Proteinase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(detection of antibodies to nonstructural 3C **proteinase** of **hepatitis A** virus)

L16 ANSWER 24 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:185013 HCAPLUS
DOCUMENT NUMBER: 126:260829
TITLE: Identification of active-site residues in **proteinase** 3C of **hepatitis A** virus by site-directed mutagenesis
AUTHOR(S): Gosert, Rainer; Dollenmaier, Guenter; Weitz, Manfred
CORPORATE SOURCE: Inst. Clin. Microbiol. Immunol., St. Gallen, CH-9001, Switz.
SOURCE: Journal of Virology (1997), 71(4), 3062-3068
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Picornavirus 3C proteases (3Cpro) are cysteine proteases related by amino acid sequence to trypsin-like serine proteases. Comparisons of 3Cpro of hepatitis A virus (HAV) to those of other picornaviruses have resulted in prediction of active-site residues: histidine at position 44 (H44), aspartic acid (D98), and cysteine (C172). To test whether these residues are key members of a putative catalytic triad, oligonucleotide-directed mutagenesis was targeted to 3Cpro in the context of natural polypeptide precursor P3. Autocatalytic processing of the polyprotein contg. wild-type or variant 3Cpro was tested by in vivo expression of **vaccinia** virus-HAV chimeras in an animal cell-T7 hybrid system and by in vitro translation of corresponding RNAs. Comparison with proteins present in HAV-infected cells showed that both expression systems mimicked authentic polyprotein processing. Individual substitutions of H44 by tyrosine and of C172 by glycine or serine resulted in complete loss of the virus-specific proteolytic cascade. In contrast, a P3 polyprotein in which D98 was substituted by asparagine underwent only slightly delayed processing, while an addnl. substitution of valine (V47) by glycine within putative protein 3A caused a more pronounced loss of processing. Therefore, apparently H44 and C172 are active-site constituents whereas D98 is not. The results, furthermore, suggest that substitution of amino acid residues distant from polyprotein cleavage sites may reduce proteolytic activity, presumably by altering substrate conformation.

IT **97162-88-4**, Picornain 3C
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(identification of active-site residues in **proteinase** 3C of **hepatitis A** virus by site-directed mutagenesis)

L16 ANSWER 25 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:182160 HCAPLUS
DOCUMENT NUMBER: 126:261419
TITLE: Comparison of picornaviral IRES-driven internal initiation of translation in cultured cells of different origins
AUTHOR(S): Borman, Andrew M.; Le Mercier, Philippe; Girard, Marc; Kean, Katherine M.
CORPORATE SOURCE: Unite de Virologie Moleculaire, Institut Pasteur, Paris, 75724, Fr.
SOURCE: Nucleic Acids Research (1997), 25(5), 925-932
CODEN: NARHAD; ISSN: 0305-1048
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We recently compared the efficiency of six picornaviral internal ribosome entry segments (IRESes) and the hepatitis C virus (HCV) IREs for their ability to drive internal initiation of translation in vitro. Here we present the results of a similar comparison performed in six different cultured cell lines infected with a recombinant **vaccinia** virus expressing the T7 polymerase and transfected with dicistronic plasmids. The IRESes could be divided into three groups: (i) the cardiovirus and aphthovirus IRESes (and the HCV element) direct internal initiation efficiently in all cell lines tested; (ii) the enterovirus and rhinovirus IRESes are at least equally efficient in several cell lines, but are extremely inefficient in certain cell types; and (iii) the **hepatitis A** virus IRES is incapable of directing efficient internal initiation in any of the cell lines used (including human hepatocytes). These are the same three groups found when IRESes were classified according to their activities in vitro, or according to

sequence homologies. In a mouse neuronal cell line, the poliovirus and other type I IRESes were not functional in an artificial bicistronic context. However, infectious poliovirions were produced efficiently after transfection of these cells with a genomic length RNA. Furthermore, activity of the type I IRESes was dramatically increased upon co-expression of the poliovirus 2A **proteinase**, demonstrating that while IREs efficiency may vary considerably from one cell type to another, at least in some cases viral proteins are capable of overcoming cell-specific translational defects.

L16 ANSWER 26 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:69702 HCAPLUS
DOCUMENT NUMBER: 124:112010
TITLE: Identification of hepatitis A virus non-structural protein 2B and its release by the major virus protease 3C
AUTHOR(S): Gosert, Rainer; Cassinotti, Pascal; Siegl, Guenter; Witz, Manfred
CORPORATE SOURCE: Inst. Clinical Microbiology Immunology, St Gallen, CH-9001, Switz.
SOURCE: Journal of General Virology (1996), 77(2), 247-55
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The RNA genome of hepatitis A virus (HAV) encodes a giant polyprotein that is putatively cleaved proteolytically into four structural and seven non-structural proteins. So far, most of the proposed non-structural proteins and their resp. cleavage sites have not been identified A **vaccinia** virus recombinant (vRGORF) contg. the complete HAV ORF under the control of the bacteriophage T7 promoter was used to express HAV in recombinant animal cells (BT7-H) that constitutively expressed T7 DNA-dependent RNA polymerase. A HAV-specific 27.5 kDa expression product was identified as peptide 2B. The 27.5 kDa 2B antigen was also found in HAV-infected MRC-5 cells. The N-terminal amino acid residues of the new peptide 2B are Ala-Lys-Ile-Ser-Leu-Phe and polyprotein cleavage between 2A and 2B occurred at amino acids 836-837 (Gln-Ala). Furthermore, heterologous expression in the same system of regions P1-P2 and of the protease 3C (3Cpro) gene, showed that P1-P2 polyprotein is not cleaved autocatalytically but by 3Cpro. Hence, 3Cpro is effective in cleaving the polyprotein 2A-2B junction.
IT **97162-88-4**, Protease 3C
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(identification of **hepatitis A** virus non-structural protein 2B and release by major virus protease 3C)

L16 ANSWER 27 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:945221 HCAPLUS
DOCUMENT NUMBER: 124:3379
TITLE: The proposed gene for VP1 of HAV encodes for a larger protein than that observed in HAV-infected cells and virions
AUTHOR(S): Dotzauer, Andreas; Vallbracht, Angelika; Keil, Guenther M.
CORPORATE SOURCE: Dep. of Medical Virology and Epidemiology of Virus Diseases, Univ. of Tuebingen, Tuebingen, D-72076, Germany
SOURCE: Virology (1995), 213(2), 671-5

CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The termini of hepatitis A virus (HAV) mature proteins have been assigned mainly by their homol. to other picornaviruses and their apparent electrophoretic mobility; the proposed coding sequence for VP1 is supposed to encompass 900 nucleotides from position 2208 to 3107 of the HAV genome. In order to further characterize this protein, we analyzed the in vitro and in vivo-synthesized translation products of the putative VP1 gene. CDNA coding for full-length VP1 was cloned under the control of a T7 promoter in pTF7-5; the resulting plasmid (pTF7-5/VP1) was used for both synthesis of RNA to program rabbit reticulocyte lysates and construction of a recombinant **vaccinia** virus (rv/T7-VP1). Immunoblot anal. and immuno-pptn. using antisera raised against a synthetic peptide corresponding to amino acids 13 to 33 of VP1 (13-33/VP1) led to identification of a 37-kDa protein in lysates of in vitro translated VP1 and rvv/T7-VP1-infected HFS cells, whereas a 33-kDa protein was detected with purified virions and in lysates of HAV-infected HFS cells. Because the antiserum used was directed against an amino-terminal part of VP1 and the amino terminus of VP1 is identified by sequence anal., these results show that AP1 present in the HAV virions and infected cells is shorter than previously proposed and suggest that the real carboxy terminus of VP1 is approx. 40 amino acids upstream. In order to limit the possible carboxy-terminal sites in the predicted region, we investigated in vitro synthesized translation products of a set of constructs with C-termini ending at potential cleavage sites for viral proteases 3C. The construct contg. the nucleotides from position 2208 to 3026 codes for a protein (1-273/VP1) which exhibits the same electrophoretic mobility as VP1 synthesized by HAV in vivo.
IT **97162-88-4**, Protease 3C
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(investigation of in vitro synthesized **HAV** VP1 translation products of a set of constructs with C-termini ending at potential cleavage sites for viral proteases 3C)

L16 ANSWER 28 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:882947 HCAPLUS
DOCUMENT NUMBER: 123:307546
TITLE: Identification and site-directed mutagenesis of the primary (2A/2B) cleavage site of the hepatitis A virus polyprotein: functional impact on the infectivity of HAV RNA transcripts
AUTHOR(S): Martin, Annette; Escriou, Nicolas; Chao, Shih-Fong; Girard, Marc; Lemon, Stanley M.; Wychowski, Czeslaw
CORPORATE SOURCE: Unite de Virologie Moleculaire, CNRS, Paris, 75724, Fr.
SOURCE: Virology (1995), 213(1), 213-22
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The junction between 2A and 2B proteins of the hepatitis A virus (HAV) polyprotein is processed by the virus-encoded 3C protease to liberate the precursor for capsid proteins, but details of this cleavage remain poorly defined. We identified the location of this primary cleavage by a novel approach involving expression of HAV polypeptides in eukaryotic cells via recombinant **vaccinia** viruses. A substrate polyprotein spanning

the putative HAV 2A/2B site was fused at its C-terminus to a poliovirus VP1 reporter sequence. This substrate was cleaved efficiently in trans by protease 3C derived from another recombinant **vaccinia** virus expressing a 3C precursor protein. N-terminal sequencing of the 2B-poliovirus VP1 fusion product identified the site of cleavage as the Gln836/Ala837 dipeptide, 144 residues upstream of the originally predicted site. Two mutations were introduced at the P1 position of the 2A/2B site: Gln836 .fwdarw. Asn, and Gln836 .fwdarw. Arg. Asn substitution at the P1 residue reduced the efficiency of cleavage in the **vaccinia** expression system and resulted in a small replication focus phenotype of virus rescued from infectious HAV RNA transcripts. Arg substitution abolished cleavage and was lethal to HAV replication. In addn. to identifying the site of the primary HAV polyprotein cleavage, these results shed light on the in vivo specificities of the HAV 3C protease.

IT **97162-88-4**, Protease 3C
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(a substrate polyprotein spanning the putative **hepatitis A** virus 2A/2B site was cleaved efficiently in trans by protease 3C derived from another recombinant **vaccinia** virus expressing a 3C precursor protein)

L16 ANSWER 29 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:766213 HCAPLUS
DOCUMENT NUMBER: 123:193275
TITLE: Two independent pathways of expression lead to self-assembly of the rabbit hemorrhagic disease virus capsid protein
AUTHOR(S): Sibilia, Mari; Boniotti, Maria Beatrice; Angoscini, Paola; Capucci, Lorenzo; Rossi, Cesare
CORPORATE SOURCE: Ist. Zooprofilattico Sperimentale Lombardia dell' Emilia, Brescia, 25124, Italy
SOURCE: Journal of Virology (1995), 69(9), 5812-15
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The rabbit hemorrhagic disease virus capsid protein was expressed in insect cells either as an individual protein species, from a mRNA analogous to the viral subgenomic RNA, or as part of a polyprotein that included the viral 3C-like protease and the RNA polymerase. Both pathways of expression led to the assembly of viruslike **particles** morphol. and antigenically similar to purified virus.

IT **97162-88-4**, Picornain 3C
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(two independent pathways of expression of self-assembly-dependent capsid protein from rabbit hemorrhagic disease virus)

L16 ANSWER 30 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:205214 HCAPLUS
DOCUMENT NUMBER: 122:49442
TITLE: Deduction of the 3C proteinases' fold
AUTHOR(S): Allaire, Marc; James, Michael
CORPORATE SOURCE: Dep. Biochemistry, Univ. Alberta, Edmonton, AB, T6G 2H7, Can.
SOURCE: Nature Structural Biology (1994), 1(8), 505-6
CODEN: NSBIEW; ISSN: 1072-8368

PUBLISHER: Nature Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 3C proteinases are picornaviral-encoded cysteine proteinases essential for the proper maturation of the nascent polyprotein into new infectious **particles.** This report discusses the structural similarities between the chymotrypsin-like family of serine proteinases and the 3C cysteine proteinases in relation to the three-dimensional structure of **hepatitis A** virus 3C **proteinase** (**HAV** -3C). Sequence alignment based on topog. equiv. residues indicates an 11% sequence identity of **HAV**-3C with chymotrypsin. Comparison of the predicted secondary structural elements with those obsd. in the exptl.-detd. structure, reveals that those predicted by Gorbalenya, A.E., et al., (1989) are close to the actual three-dimensional structure. The strategy adopted by Gorbalenya, A.E., et al., (1989) successfully predicted the amino terminal helix found in **HAV**-3C but not present in any of the chymotrypsin-like serine proteinases. Perfect alignment was also detected in regions involving the nucleophilic residue (**HAV**-3C:Cys 172), the general base residue (**HAV**-3C:His 44), and the residues involved in defining the S1 specificity pocket (**HAV**-3C:His 191). Sequence alignment was also perfect around **HAV**-3C:Asp 84, and in regions that do not have conserved active site residues which help in defining the sequence alignment (COOH-terminal helix; .beta.-strands A2, B2 and F2).
IT **97162-88-4**, 3C **Proteinase**
RL: PRP (Properties)
(deduction of 3C proteinases' fold based on structural comparison chymotrypsin-like family of serine proteinases)

L16 ANSWER 31 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:431034 HCAPLUS
DOCUMENT NUMBER: 121:31034
TITLE: Cloning of a cDNA for the genome of a Norwalk virus and its use in the diagnosis of infection by Norwalk and related viruses
INVENTOR(S): Matson, David O.; Estes, Mary K.; Jiang, Xi; Graham, David Y.
PATENT ASSIGNEE(S): Baylor College of Medicine, USA
SOURCE: PCT Int. Appl., 157 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9405700	A2	19940317	WO 1993-US8447	19930907
WO 9405700	A3	19940804		
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9348514	A1	19940329	AU 1993-48514	19930907
JP 08500250	T2	19960116	JP 1993-507522	19930907
PRIORITY APPLN. INFO.:			US 1992-941365 A	19920907
			WO 1993-US8447 W	19930907

AB CDNAs for the genome of Norwalk virus are cloned for use in the detection of the virus in the diagnosis of acute viral gastroenteritis. The availability of a Norwalk-specific cDNA and the genome sequence information allow rapid cloning of the entire genome and establishment of

sensitive diagnostic assays. These assays can use nucleic acid hybridization, PCR, or immunoassays using antibodies against antigenic peptides identified from the cloned cDNA and synthesized chem. or by expression of the cloned sequence. These antigens can also be used in **vaccines**. Double-stranded cDNA was synthesized from nucleic acid extd. from Norwalk virus purified from stool specimens of volunteers. Single-stranded RNA probes derived from the DNA clone after subcloning into an in vitro transcription vector were also used to show that the Norwalk virus contains an ssRNA genome of about 8 kb.

IT **97162-88-4**, Picornain 3C
RL: BIOL (Biological study)
(Norwalk virus homolog of, gene for, cloning of)

L16 ANSWER 32 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:428252 HCAPLUS
DOCUMENT NUMBER: 121:28252
TITLE: Construction of a recombinant cDNA of echovirus 6 that established a persistent in vitro infection
AUTHOR(S): Gratsch, Theresa E.; Righthand, V. Fay
CORPORATE SOURCE: Sch. Med., Wayne State Univ., Detroit, MI, 48201, USA
SOURCE: Virology (1994), 201(2), 341-8
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CDNA clones of lytic acute and nonlytic persistent strains of echovirus 6 were used to construct a recombinant cDNA. The 3' region of the infectious wild-type cDNA genome, which extended from VPg to the end of the noncoding region, was exchanged with the cDNA fragment representing the same region of the persistent viral genome. Sequence analyses indicated that there was one mutation in the 3C protease and eight mutations in the 3D polymerase. Transfection of the recombinant cDNA into WISH cells resulted in cellular survival and synthesis of viral RNA. The viral RNA was retained in the transfected cell line after cultivation for 7 mo. Supernates, collected from cell cultures at 1, 3, and 7 mo after transfection with the recombinant cDNA, transmitted the viral RNA to uninfected cells. The results indicated that the recombinant cDNA established a persistent echovirus 6 infection that was transmissible by nonlytic virus **particles**.

IT **97162-88-4**, 3C **Proteinase**
RL: PRP (Properties)
(mutation of gene for, in persistent infection by echovirus 6, recombinant cDNA construction for study of)

L16 ANSWER 33 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:428088 HCAPLUS
DOCUMENT NUMBER: 121:28088
TITLE: Attenuated poliovirus strain as a live vector: expression of regions of rotavirus outer capsid protein VP7 by using recombinant Sabin 3 viruses
AUTHOR(S): Mattion, Nora M.; Reilly, Patricia A.; DiMichele, Susan J.; Crowley, Joan C.; Weeks-Levy; Carolyn
CORPORATE SOURCE: Lederle-Praxis Biol., Pearl River, NY, 10965, USA
SOURCE: Journal of Virology (1994), 68(6), 3925-33
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ability to express heterologous antigens from attenuated poliovirus strains suggests the potential for use as live vectored **vaccines**. Full- or partial-length sequences of the gene encoding rotavirus major

outer capsid protein VP7 were cloned into the open reading frame of a full-length cDNA copy of poliovirus Sabin type 3. They were inserted either at the 5' end or immediately after the capsid protein coding region, at the junction between precursors P1 and P2. A protease cleavage site for 3C protease was introduced 3' to the foreign sequences to enable proteolytic processing of the antigen from the poliovirus polyprotein. Infectious viruses were generated from several of the DNA constructs, and the presence of the foreign gene sequences was confirmed by reverse transcription of the viral RNA and PCR amplification. Viruses with inserts of about 300 bases maintained the foreign sequences during passage in Vero cells. Viruses carrying larger sequences were unstable, and deletions were generated within the foreign sequences. Expression of the VP7 polypeptides was demonstrated by immunopptn. with specific antiserum of labeled proteins from cells infected with Sabin 3 recombinant viruses. Comparative studies of RNA synthesis showed similar kinetics for Sabin 3 and the Sabin 3/VP7 recombinants. One-step growth curves showed that prodn. of recombinant viruses was slower than that of Sabin 3 and that the final titers were 1 to 1.5 logs lower. Accumulation of VP7-contg. precursors in infected cells suggests that slow cleavage at the engineered 3C protease site may be a limiting step in the growth of these recombinant Sabin polioviruses and may influence the permissible size of foreign sequence to be inserted.

IT **97162-88-4**, 3C Protease
RL: BIOL (Biological study)
(rotavirus VP7 proteolytic processing from poliovirus polyprotein by, rotavirus **vaccine** development in relation to)

L16 ANSWER 34 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:237609 HCAPLUS
DOCUMENT NUMBER: 120:237609
TITLE: Manufacture of hepatitis A virus **particles** in a baculovirus system for **vaccines**
INVENTOR(S): McLinden, James H.; Rosen, Elliot D.; Winokur, Patricia L.; Stapleton, Jack T.
PATENT ASSIGNEE(S): American Biogenetic Sciences, Inc., USA; University of Iowa Research Foundation
SOURCE: U.S., 36 pp. Cont.-in-part of U.S. Ser. No. 502,900, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5294548	A	19940315	US 1991-725178	19910703
WO 9301279	A1	19930121	WO 1992-US5714	19920702
W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KR, LK, MG, MN, NO, PL, RO, RU, SD				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
AU 9223716	A1	19930211	AU 1992-23716	19920702

PRIORITY APPLN. INFO.: US 1990-502900 19900402
US 1991-725178 19910703
WO 1992-US5714 19920702

AB A method of manufg. non-infectious hepatitis A virus (HAV) capsid **particles** by heterologous expression of the genes is described. The method is demonstrated using a baculovirus system. These

baculoviruses are formed by replacing regions of the polyhedrin structural gene with HAV DNA by in vivo recombination. The polyhedrin transcription start site is altered in these baculoviruses to ensure that only HAV proteins and not polyhedrin protein sequences are expressed from the polyhedrin promoter. Expression of the gene for the VP1-VP4 polyprotein in a baculovirus system led to the formation of capsid-like structures that could be banded in CsCl d. gradients. The immunoreactive fraction reacted with antibodies to VP1 and identified a no. of VP1-contg. processing intermediates in the capsid fraction.

IT **9001-92-7**, Protease
RL: BIOL (Biological study)
(polyprotein (3C), of **hepatitis A** virus, manuf. in animal cell culture of, for processing of viral polyprotein, viral expression vectors for, **vaccines** in relation to)

L16 ANSWER 35 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:4311 HCAPLUS
DOCUMENT NUMBER: 120:4311
TITLE: Expression of poliovirus P3 proteins using a recombinant **vaccinia** virus results in proteolytically active 3CD precursor protein without further processing to 3Cpro and 3Dpol
AUTHOR(S): Porter, Donna C.; Ansardi, David C.; Lentz, Michael R.; Morrow, Casey D.
CORPORATE SOURCE: Dep. Microbiol., Univ. Alabama, Birmingham, AL, 35294, USA
SOURCE: Virus Research (1993), 29(3), 241-54
CODEN: VIREDF; ISSN: 0168-1702
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The expression of the poliovirus genome occurs by the translation of a single open reading frame to generate a long polyprotein which is subsequently processed by viral encoded proteases. The initial proteolytic cleavages result in the prodn. of a P1 polyprotein which contains the capsid proteins, and the P2 and P3 polyproteins which contain proteins required for replication. The P3 polyprotein consists of the 3AB protein (contg. the viral genome-linked protein, VPg), the viral protease, 3Cpro, and RNA polymerase, 3Dpol. To further study the expression and proteolytic processing of poliovirus P3 proteins in vivo, the authors have utilized recombinant **vaccinia** virus vectors to express nucleotides 5240-7400 contg. the P3 region proteins of poliovirus. The P3 protein expressed from the recombinant **vaccinia** virus VV-P3 exhibited in vivo proteolytic activity as evident by processing of the polyprotein to generate the 3CD protein, consisting of a fusion between the 3Cpro and 3Dpol proteins. Further processing of the 3CD protein to 3Cpro and 3Dpol, however, was not detected in cells infected with VV-P3. Subcellular fractionation of VV-P3-infected cells demonstrated that the 3CD protein was present in both the sol. and membrane fractions. Finally, the 3CD protein expressed from VV-P3 was stable in cells coinfected with VV-P3 and poliovirus and no further processing to 3Dpol was detected. Thus, the 3CD polyprotein is not a precursor to 3Dpol in poliovirus-infected cells.

IT **97162-88-4**, Protease 3C
RL: FORM (Formation, nonpreparative)
(formation of, in poliovirus-infected cells, P3 polyprotein processing in relation to)

L16 ANSWER 36 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1993:510582 HCAPLUS

DOCUMENT NUMBER: 119:110582
TITLE: Recombinant viruses comprising artificial proteolytic cleavage site and their use in **vaccines**
INVENTOR(S): Feinberg, Mark; Andino, Raul; Weeks-Levy, Carolyn Louise; Reilly, Patricia Anne
PATENT ASSIGNEE(S): Whitehead Institute for Biomedical Research, USA; American Cyanamid Co.
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311251	A1	19930610	WO 1992-US10543	19921204
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
ZA 9209418	A	19930607	ZA 1992-9418	19921204
AU 9332424	A1	19930628	AU 1993-32424	19921204
AU 674134	B2	19961212		
CN 1075334	A	19930818	CN 1992-115173	19921204
CN 1055726	B	20000823		
JP 07502403	T2	19950316	JP 1992-510379	19921204
HU 67346	A2	19950328	HU 1994-1689	19921204
EP 672157	A1	19950920	EP 1993-900925	19921204
EP 672157	B1	20011010		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
AT 206762	E	20011015	AT 1993-900925	19921204
ES 2163405	T3	20020201	ES 1993-900925	19921204
TW 403784	B	20000901	TW 1993-82101918	19930316
FI 9402623	A	19940721	FI 1994-2623	19940603
NO 9402075	A	19940803	NO 1994-2075	19940603
US 5965124	A	19991012	US 1995-381637	19950131

PRIORITY APPLN. INFO.:
US 1991-804893 A 19911206
US 1992-947790 A 19920918
WO 1992-US10543 A 19921204
US 1992-986729 B1 19921208

AB Replication-competent recombinant viruses encoding an exogenous protein linked to the viral polyprotein through 1 or 2 artificial proteolytic cleavage sites are claimed. When the viral polyprotein is cleaved during viral infection, the heterologous protein is released also. These recombinant viruses can be used as **vaccines** if the exogenous protein is an antigen from a pathogen. Recombinant poliovirus encoding cholera toxin B subunit fused to the amino terminal of the polyprotein through a poliovirus 3C protease cleavage site was prepd. HeLa cells infected with this virus produced a larger than normal polyprotein which was proteolytically processed to produce normal viral proteins as well as the cholera toxin subunit B.

IT **97162-88-4**, Picornain 3C
RL: BIOL (Biological study)
(cleavage site for, replication-competent recombinant poliovirus encoding antigen linked to polyprotein by, immunization in relation to)

L16 ANSWER 37 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1993:189960 HCAPLUS

DOCUMENT NUMBER: 118:189960
TITLE: Recombinant hepatitis A virus **vaccine**
INVENTOR(S): McLinden, James H.; Rosen, Elliot D.; Stapleton, Jack T.; Winokur, Patricia L.
PATENT ASSIGNEE(S): American Biogenetic Sciences, Inc., USA; University of Iowa Research Foundation
SOURCE: PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9301279	A1	19930121	WO 1992-US5714	19920702
W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KR, LK, MG, MN, NO, PL, RO, RU, SD				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
US 5294548	A	19940315	US 1991-725178	19910703
AU 9223716	A1	19930211	AU 1992-23716	19920702

PRIORITY APPLN. INFO.: US 1991-725178 19910703
US 1990-502900 19900402
WO 1992-US5714 19920702

AB A recombinant virus, e.g. a baculovirus or **vaccinia** virus, is disclosed which contains all coding regions of hepatitis A virus (HAV) polyprotein controlled by regulatory elements such that HAV polyprotein is expressed and processed into capsid proteins. The viral genome may encode the P1 precursor protein and the protease polymerase polyprotein (or an activity-increasing mutation thereof) of HAV. Thus, Spodoptera frugiperda cells were infected with recombinant baculovirus A contg. the HAV polyprotein gene under the control of the polyhedrin promoter. Lysates of the cells harvested 4 days later contained HAV polyprotein processing intermediates and products including capsid proteins VP1, VP2, VP3, and VP4.

IT **9001-92-7, Proteinase**
RL: BIOL (Biological study)
(of **hepatitis A** virus polyprotein, recombinant, as **vaccine**)

L16 ANSWER 38 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:610626 HCAPLUS
DOCUMENT NUMBER: 111:210626
TITLE: Processing and assembly of foot-and-mouth disease virus proteins using subgenomic RNA
AUTHOR(S): Clarke, B. E.; Sangar, D. V.
CORPORATE SOURCE: Dep. Virol., Wellcome Biotechnol. Ltd., Beckenham/Kent, BR3 3BS, UK
SOURCE: Journal of General Virology (1988), 69(9), 2313-25
CODEN: JGVIAY; ISSN: 0022-1317
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant DNA clones were constructed to study the mechanisms of proteolytic processing and assembly in foot-and-mouth disease virus (FMDV). RNA transcripts from these clones were synthesized using SP6 polymerase and translated in rabbit reticulocyte lysates. Efficient translation occurred in the absence of all 5' untranslated sequences and processing of the structural proteins occurred in the presence of

functional 3C protease which can function in trans. The specificity of 3C protease activity is not limited to Glu-Gly bonds. Translation of correctly processed structural proteins leads to assembly of subviral structures resembling empty **particles**. Further studies on the processing of the FMDV genome show that the primary cleavage (P1-P2) is mediated neither by 3C nor the 2nd FMDV protease L. Preliminary evidence suggests that an initial very rapid cleavage occurs between 2A and 2B with subsequent cleavage of the P1/2A junction probably being carried out by 3C.

IT **97162-88-4**
RL: BIOL (Biological study)
(in processing of foot-and-mouth disease virus proteins)

L16 ANSWER 39 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:129894 HCAPLUS
DOCUMENT NUMBER: 110:129894
TITLE: Recombinant production of hepatitis A virus proteins and their use as **vaccines**
INVENTOR(S): Ovchinnikov, Yu. A.; Sverdlov, E. D.; Tsarev, S. An.; Frolova, E. I.; Rokhlina, T. O.; Rostapshov, V. M.; Azhikina, T. L.; Arsenyan, S. G.; Snezhkov, E. V.; et al.
PATENT ASSIGNEE(S): Shemyakin, M. M., Institute of Bioorganic Chemistry, USSR; Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences, U.S.S.R.; All-Union Scientific-Research Institute of Molecular Biology; Moscow Scientific-Research Institute of Viral Preparations
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Russian
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8800973	A1	19880211	WO 1987-SU85	19870731
W: JP, US				
RW: CH, DE, FR, GB				
SU 1469856	A1	19900930	SU 1986-4102079	19860731
EP 276330	A1	19880803	EP 1987-905562	19870731
R: CH, DE, FR, GB, LI				
JP 01500485	T2	19890223	JP 1987-504983	19870731
PRIORITY APPLN. INFO.:			SU 1986-4102079	19860731
			WO 1987-SU85	19870731

AB Plasmid vectors contg. a DNA fragment encoding **hepatitis A** virus proteins VP1, VP3, VP4 into the nonstructural protein region or the latter fused to a sequence encoding viral **proteinase**, or a synthetic DNA sequence corresponding to amino acids (aa) 11-25 of VP1 and either a marker galactosidase gene of Escherichia coli or a human .gamma.-interferon gene were constructed. Vectors were amplified and expressed in E. coli or used to transform CV1 cells that had been infected with **vaccinia** virus. **Vaccinia** virus recombinants contg. the **hepatitis A** virus protein-encoding sequence were recovered from the latter and used to transfect RAT-2 cells. Hepatitis virus proteins expressed in E. coli or RAT-2 cells were recovered after cell lysis and used to immunize guinea pigs or rabbits. The PstI fragment of plasmid pHAV23 contg. a 3372 bp sequence encoding

hepatitis A virus protein VP4 from aa 38 into the nonstructural protein region was inserted into the polylinker in plasmid pSPVV. The recombinant plasmid pSP-VV-**HAV**-D was amplified in E. coli, recovered, and used to transform **vaccinia** virus-infected CV1 cells. The viral **particles** obtained were propagated on a RAT2 cell culture and the **hepatitis A** proteins in a lysate prepn. (5 x 109 **particles**/mL) were used to immunize rabbits. After 3-4 days a specific inflammatory reaction was elicited and after 3-4 wk the blood serum of the treated rabbits showed anti-**vaccinia** virus titers of 1:640, 1:1280. The serum also contained **hepatitis A** virus antibody.

L16 ANSWER 40 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1987:2427 HCAPLUS
DOCUMENT NUMBER: 106:2427
TITLE: Detection of hepatitis A virus in seeded estuarine samples by hybridization with cDNA probes
AUTHOR(S): Jiang, Xi; Estes, Mary K.; Metcalf, Theodore G.; Melnick, Joseph L.
CORPORATE SOURCE: Dep. Virol. Epidemiol., Baylor Coll. Med., Houston, TX, 77030, USA
SOURCE: Applied and Environmental Microbiology (1986), 52(4), 711-17
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The development and trials of a nucleic acid hybridization test for the detection of **hepatitis A** virus (**HAV**) in estuarine samples within 48 h are described. Approx. 104 phys. **particles** of **HAV** per dot could be detected. Test sensitivity was optimized by the consideration of hybridization stringency, 32P energy level, probe concn., and nucleic acid binding to filters. Test specificity was shown by a lack of cross-hybridization with other enteroviruses and unrelated nucleic acids. Potential false-pos. reactions between bacterial DNA in samples and residual vector DNA contamination of purified nucleotide sequences in probes were eliminated by **DNase** treatment of samples. Humic acid at .ltoreq.100 mg/L caused only insignificant decreases in test sensitivity. Interference with hybridization by org. components of virus-contg. eluates was removed by **proteinase** K digestion followed by PhOH extn. and EtOH pptn. The test is suitable for detecting naturally occurring **HAV** in samples from polluted estuarine environments.
IT **39450-01-6**
RL: ANST (Analytical study)
(in **hepatitis A** virus detection by cDNA hybridization probe, in polluted estuarine environment samples)

L16 ANSWER 41 OF 41 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1978:503320 HCAPLUS
DOCUMENT NUMBER: 89:103320
TITLE: Biochemical and biophysical characterization of light and heavy density hepatitis A virus **particles** : evidence HAV is an RNA virus
AUTHOR(S): Bradley, Daniel W.; Fields, Howard A.; McCaustland, Karen A.; Cook, E. H.; Gravelle, Clifton R.; Maynard, James E.
CORPORATE SOURCE: Public Health Serv., U. S. Dep. Health, Educ., Welfare, Phoenix, AZ, USA
SOURCE: Journal of Medical Virology (1978), 2(2), 175-87

Searched by M. Smith

CODEN: JMVIDB; ISSN: 0146-6615
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Light d. (1.34 g/cm3) and heavy d. (1.45 g/cm3) **hepatitis A** virus (**HAV**) **particles** had identical sedimentation coeffs. of .apprx.157 S in neutral sucrose gradients. Heavy d. **HAV** sedimented at 157 and 230 S in linear sucrose gradients contg. 1.5 M CsCl, while light d. **HAV** sedimented only at 157 S. Alk. pH degrdn. of light d. **HAV** revealed losses of 50 and 100% of 157 S virus at pH 10.0 and 11.0, resp. Alk. pH treatment of heavy d. **HAV** yielded a dissimilar degrdn. profile: a considerable proportion of 157 S antigen was not lost after pH 11.0 treatment. Light d. **HAV** treated at pH 10.0 was very sensitive to RNase but not to **DNase**. Heavy d. **HAV** was also sensitive to low concns. of RNase. Thus, **HAV** is probably an enterovirus.